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L6: Entry 4 of 5 File: USPT Apr 24, 2001

DOCUMENT-IDENTIFIER: US 6221349 B1
TITLE: Adeno-associated vectors for expression of factor VIII by target cells

Detailed Description Text (13):
As used herein, the term "AAV vector" refers to a vector having functional or partly functional ITR sequences. The ITR sequences may be derived from an adeno-associated virus serotype, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-X7, etc. The ITRs, however, need not be the wild-type nucleotide sequences, and may be altered (e.g., by the insertion, deletion or substitution of nucleotides), so long as the sequences retain function provide for functional rescue, replication and packaging. AAV vectors can have one or more of the AAV wild-type genes deleted in whole or part, preferably the rep and/or cap genes but retain functional flanking ITR sequences. Functional ITR sequences are necessary for the rescue, replication and packaging of the AAV virion. Thus, an "AAV vector" is defined herein to include at least those sequences required in cis for replication and packaging (e.g., functional ITRs) of the virus.

Detailed Description Text (48):
Adeno-associated virus (AAV) is a non-pathogenic, replication-defective, helper-dependent parvovirus (or "dependovirus" or "adeno-satellite virus"). There are at least six recognized serotypes, designated as AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-X7, etc. Culture and serologic evidence indicates that human infection occurs with AAV-2 and AAV-3. Although 85% of the human population is seropositive for AAV-2, the virus has never been associated with disease in humans. Recombinant AAV (rAAV) virions are of interest as vectors for gene therapy because of their broad host range, excellent safety profile, and duration of transgene expression in infected hosts. One remarkable feature of recombinant AAV (rAAV) virions is the prolonged expression achieved after in vivo administration.

Detailed Description Text (50):
The nucleotide sequences of AAV ITR regions are known (See e.g., Kotin, Hum. Gene Ther., 5:793-801 [1994]; Berns, "Parvoviridae and Their Replication" in Fields and Knipe (eds), Fundamental Virology, 2nd Edition, for the AAV-2 sequence). AAV ITRs used in the vectors of the invention need not have a wild-type nucleotide sequence, and may be altered (e.g., by the insertion, deletion or substitution of nucleotides). Additionally, AAV ITRs may be derived from any of several AAV serotypes, including without limitation, AAV-1, AAV-2,

AAV-3, AAV-4, AAV-5, AAVX7, etc. Furthermore, 5' and 3' ITRs which flank a selected nucleotide sequence in an AAV vector need not necessarily be identical or derived from the same AAV serotype or isolate, so long as they function as intended.

Other Reference Publication (68):
Kaplitt et al., "Viral Vectors for Gene Delivery and Expression in the CNS," in Methods: A Companion to Methods in Enzymology 10: 343-350 (1996).

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L13: Entry 1 of 2 File: USPT Mar 27, 2001

DOCUMENT-IDENTIFIER: US 6207457 B1
TITLE: Targeted nucleotide sequence delivery and integration system

Detailed Description Text (9):
For the purpose of the present invention, a single AAV ITR is positioned relative to one or more selected heterologous nucleotide sequences and, together with an expressible AAV rep coding region or Rep expression products, can provide for the integration of the associated sequences into the genome of a target cell. The nucleotide sequences of AAV ITR regions are known. See, e.g., Kotin, R. M. (1994) Human Gene Therapy 5:793-801; Berns, K. I. "Parvoviridae and their Replication" in Fundamental Virology, 2nd Edition, (B. N. Fields and D. M. Knipe, eds.), see FIG. 1 (SEQ. ID No. 1) for the AAV-2 sequence. As used herein, an "AAV ITR" need not have the wild-type nucleotide sequence depicted, but may be altered, e.g., by the insertion, deletion or substitution of nucleotides. Additionally, the AAV ITR may be derived from any of several AAV serotypes, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAVX7, etc. However, the term "AAV ITR" as used herein denotes a nucleotide sequence having not more than one region that is substantially homologous to the 20 base "D" region found in wild-type AAV ITR sequences. The AAV ITR sequence need only function as intended, i.e., to allow for the integration of the associated heterologous sequence into the recipient cell genome when an expressible AAV rep coding region is present (either on the same or on a different vector), or when Rep expression products have also been introduced into the recipient cell.

Detailed Description Text (39):
In one particular aspect of the invention, an isolated nucleic acid construct is provided having a targeting sequence that is homologous to a single AAV ITR sequence. As described above, AAV ITRs are approximately 145 base nucleotide sequences that comprise three palindromic sequences, denoted "A," "B" and "C" (Lusby et al. (1980) J. Virol. 34:402-409; Straus et al. (1976) Proc. Natl. Acad. Sci. USA 73:742-746), and a single 3' 20 nucleotide non-palindromic region denoted "D." Included in the ITR sequence is a functional Rep binding site as described above. Under the invention, the ITR sequence need not be a wild-type sequence, e.g., it can be altered such as by the insertion, deletion or substitution of nucleotides. The ITR can be synthetically derived using known methods, and/or the ITR may be derived from any of several AAV serotypes, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAVX7, etc. However, the ITR is selected so as to have not more than one region that is substantially homologous to the 20 base "D" region found in

wild-type AAV ITR sequences. In one particular construct, the targeting sequence comprises the 145 base pair ITR sequence as depicted in FIG. 1 (SEQ ID NO: 1).

Detailed Description Text (63):
Each of the present methods of integrating a selected nucleotide sequence into a recipient cell genome entails introducing genetic information along with Rep expression products into a suitable recipient cell, whereby selected nucleotide sequences of interest are site-specifically integrated into the recipient cell genome. Thus, once engineered and assembled, the present integration systems can be used directly to transform a selected recipient cell. In this regard, the cell to be transformed will depend on the purpose for gene transfer, e.g., the disease state being treated. For example, the nucleotide integration system of the present invention can be used to deliver and integrate nucleotide sequences into any nucleated cell including stem, progenitor and erythroid cells; as well as any of the various white blood cells such as lymphocytes, neutrophils, eosinophils, basophils, monocytes; tissue specific cells, such as those derived from lung, heart, kidney, liver, spleen, pancreatic tissue, connective tissue, muscle and bone tissue including osteocytes, gangliocytes, epithelial and endothelial cells, ependymal cells, reticuloendothelial cells, dendritic and neural cells, and the like.

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End of Result Set -Does not use an AAV particle; their method uses a virion-free nucleic acid construct.

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L1: Entry 3 of 3 File: USPT Dec 1, 1998

DOCUMENT-IDENTIFIER: US 5843742 A
TITLE: Adeno-associated derived vector systems for gene delivery and integration into target cells

Detailed Description Text (8):
The nucleotide sequences of AAV ITR regions are known. See, e.g., Kotin, R. M. (1994) Human Gene Therapy 5:793-801; Bems, K. I. "Parvoviridae and their Replication" in Fundamental Virology, 2nd Edition, (B. N. Fields and D. M. Knipe, eds.) for the AAV-2 sequence. As used herein, an "AAV ITR" need not have the wild-type nucleotide sequence depicted, but may be altered, e.g., by the insertion, deletion or substitution of nucleotides. Additionally, the AAV ITR may be derived from any of several AAV serotypes, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAVX7, etc. The 5' and 3' ITRs which flank a selected heterologous nucleotide sequence need not necessarily be identical or derived from the same AAV serotype or isolate, so long as they function as intended, i.e., to allow for the integration of the associated heterologous sequence into the target cell genome when the rep gene is present (either on the same or on a different vector), or when the Rep expression product is present in the target cell.

Detailed Description Text (10):
By an "AAV vector" is meant a vector derived from an adeno-associated virus serotype, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAVX7, etc. AAV vectors can have one or more of the AAV wild-type genes deleted in whole or part, preferably the rep and/or cap genes, but retain functional flanking ITR sequences.

Detailed Description Text (44):
Once engineered, the constructs can be used directly to transform a selected target cell. In this regard, the cell to be transformed will depend on the purpose for gene transfer, e.g., the disease state being treated. For example, the system of the present invention can be used to deliver and integrate nucleotide sequences into any nucleated cell including stem, progenitor and erythroid cells; as well as any of the various white blood cells such as lymphocytes, neutrophils, eosinophils, basophils, monocytes; tissue specific cells, such as those derived from lung, heart, kidney, liver, spleen, pancreatic tissue, connective tissue, muscle and bone tissue including osteocytes, gangliocytes, epithelial and endothelial cells, ependymal cells, reticuloendothelial cells, dendritic and neural cells, and the like.

WEST Search History

DATE: Sunday, September 29, 2002

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT; PLUR=YES; OP=ADJ		
L15	6211163.pn.	1	L15
L14	L11 and cerebell$	0	L14
L13	L11 and ependyma$	2	L13
L12	L11 and alveola$	3	L12
L11	AAV5 or AAV-5 or AAV 5	40	L11
L10	AAV and alveola$	51	L10
L9	6180613.pn. and AAV	1	L9
L8	6180613.pn.	1	L8
L7	6180613.pn. and (AAV5 or AAV 5 or AAV-5)	0	L7
L6	L3 and CNS	5	L6
L5	L3 and AAV-5	28	L5
L4	L3 and L2 and L1	0	L4
L3	AAV 5	38	L3
L2	AAV-5	28	L2
L1	AAV5	2	L1

9/29/02 AMJ

END OF SEARCH HISTORY

Amj

WEST

End of Result Set

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L13: Entry 2 of 2 File: USPT Dec 1, 1998

DOCUMENT-IDENTIFIER: US 5843742 A
TITLE: Adeno-associated derived vector systems for gene delivery and integration into target cells

Detailed Description Text (8):
The nucleotide sequences of AAV ITR regions are known. See, e.g., Kotin, R. M. (1994) Human Gene Therapy 5:793-801; Bems, K. I. "Parvoviridae and their Replication" in Fundamental Virology, 2nd Edition, (B. N. Fields and D. M. Knipe, eds.) for the AAV-2 sequence. As used herein, an "AAV ITR" need not have the wild-type nucleotide sequence depicted, but may be altered, e.g., by the insertion, deletion or substitution of nucleotides. Additionally, the AAV ITR may be derived from any of several AAV serotypes, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAVX7, etc. The 5' and 3' ITRs which flank a selected heterologous nucleotide sequence need not necessarily be identical or derived from the same AAV serotype or isolate, so long as they function as intended, i.e., to allow for the integration of the associated heterologous sequence into the target cell genome when the rep gene is present (either on the same or on a different vector), or when the Rep expression product is present in the target cell.

Detailed Description Text (10):
By an "AAV vector" is meant a vector derived from an adeno-associated virus serotype, including without limitation, AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAVX7, etc. AAV vectors can have one or more of the AAV wild-type genes deleted in whole or part, preferably the rep and/or cap genes, but retain functional flanking ITR sequences.

Detailed Description Text (44):
Once engineered, the constructs can be used directly to transform a selected target cell. In this regard, the cell to be transformed will depend on the purpose for gene transfer, e.g., the disease state being treated. For example, the system of the present invention can be used to deliver and integrate nucleotide sequences into any nucleated cell including stem, progenitor and erythroid cells; as well as any of the various white blood cells such as lymphocytes, neutrophils, eosinophils, basophils, monocytes; tissue specific cells, such as those derived from lung, heart, kidney, liver, spleen, pancreatic tissue, connective tissue, muscle and bone tissue including osteocytes, gangliocytes, epithelial and endothelial cells, ependymal cells, reticuloendothelial cells, dendritic and neural cells, and the like.

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End of Result Set

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L9: Entry 1 of 1 File: USPT Jan 30, 2001

DOCUMENT-IDENTIFIER: US 6180613 B1
TITLE: AAV-mediated delivery of DNA to cells of the nervous system

US PATENT NO. (1):
6180613

Abstract Text (1):
The invention relates to a method of delivering exogenous DNA to a target cell of the mammalian central nervous system using an adeno-associated virus (AAV)-derived vector. Also included in the invention are the AAV-derived vectors containing exogenous DNA which encodes a protein or proteins which treat nervous system disease, and a method of treating such disease.

Brief Summary Text (24):
Adeno-Associated Virus is a defective parvovirus whose genome is encapsidated as a single-stranded DNA molecule. Strands of plus and minus polarity are both packaged, but in separate virus particles. Although AAV can replicate under special circumstances in the absence of a helper virus, efficient replication requires coinfection with a helper virus of the herpesvirus or adenovirus family. In the absence of the helper virus, AAV establishes a latent infection in which the viral genome exists as an integrated provirus in the host cell. (No AAV gene expression is required to establish a latent infection). The integration of the virus is site-specific (chromosome 19). If a latently infected cell line is later superinfected with a suitable helper virus, the AAV provirus is excised and the virus enters the "productive" phase of its life cycle. However, it has been reported that certain AAV-derived transducing vectors are not rescued by adenovirus superinfection.

Brief Summary Text (25):
Although AAV is a human virus, its host range for lytic growth is unusually broad. Cell lines from virtually every mammalian species tested (including a variety of human, simian, canine, bovine and rodent cell lines) can be productively infected with AAV, provided an appropriate helper virus is used (e.g., canine adenovirus in canine cells). Despite this, no disease has been associated with AAV in either human or other animal populations, unlike both HSV and adenovirus.

Brief Summary Text (26):
AAV has been isolated as a nonpathogenic coinfecting agent from fecal, ocular and respiratory specimens during acute adenovirus

infections, but not during other illnesses.

Brief Summary Text (27):
Likewise, latent AAV infections have been identified in both human and nonhuman cells. Overall, virus integration appears to have no apparent effect on cell growth or morphology. See Samulski (1993) Curr. Op. Gen. Devel. 3:74-80.

Brief Summary Text (28):
The genome of AAV-2 is 4,675 bases in length and is flanked by inverted terminal repeat sequences of 145 bases each. These repeats are believed to act as origins for DNA replication.

Brief Summary Text (30):
The right ORF, controlled by the P40 promoter, encodes the capsid proteins Vp1 (91 kDa), Vp2 (72 kDa) and Vp3 (60 kDa). Vp3 comprises 80% of the virion structure, while Vp1 and Vp2 are minor components. There is a polyadenylation site at map unit 95. For the complete sequence of the AAV-2 genome, see Vastava et al (1983) J. Virol. 45:555-64.

Brief Summary Text (31):
McLaughlin et al ((1988) J. Virol. 62:1963-73) prepared two AAV vectors: dl 52-91, which retains the AAV rep genes, and dl 3-94, in which all of the AAV coding sequences have been deleted. It does, however, retain the two 145 base terminal repeats, and an additional 139 bases which contain the AAV polyadenylation signal. Restriction sites were introduced on either side of the signal.

Brief Summary Text (32):
A foreign gene, encoding neomycin resistance, was inserted into both vectors. Viral stocks were prepared by complementation with a recombinant AAV genome, which supplied the missing AAV gene products in trans but was itself too large to be packaged.

Brief Summary Text (33):
Unfortunately, the virus stocks were contaminated with wild type AAV (10% in the case of dl 3-94) presumably as a result of homologous recombination between the defective and the complementing virus.

Brief Summary Text (34):
Samulski et al ((1989) J. Virol. 63:3822-28) developed a method of producing recombinant AAV stocks without detectable wild-type helper AAV. Their AAV vector retained only the terminal 191 bases of the AAV chromosome. In the helper AAV, the terminal 191 bases of the AAV chromosome were replaced with adenovirus terminal sequences. Since sequence homology between the vector and the helper AAV was thus essentially eliminated, no detectable wild-type AAV was generated by homologous recombination. Moreover, the helper DNA itself was not replicated and encapsidated because the AAV termini are required for this process. Thus, in the AAV system, unlike the HSV system, helper virus could be completely eliminated leaving a helper-free AAV vector stock.

Brief Summary Text (35):
Muro-Cacho et al ((1992) J. Immunother. 11:231-237) have used

AAV-based vectors for gene transfer into both T- and B-lymphocytes. Walsh et al ((1992) Proc. Nat. Acad. Sci. (USA) 89:7257-61) used an AAV vector to introduce a human gamma globulin gene into human erythroleukemia cells; the gene was expressed. Flothe et al ((1993) J. Biol. Chem. 268:3781-90) delivered the cystic fibrosis transmembrane conductance regulator gene to airway epithelial cells by means of an AAV vector. See also Flotte et al (1992) Am. J. Respir. Cell. Mol. Biol. 7:349-56; Flotte et al (1993) Proc. Nat. Acad. Sci. (USA) 90:10613-17.

Brief Summary Text (37):
Adeno-associated virus has not been reported to naturally infect any nervous system cells, and AAV-derived vectors have not previously been used to transfect terminally differentiated, non-dividing cells. Nonetheless, the present invention demonstrates that an adeno-associated virus-derived vector may be used to deliver exogenous DNA to cells of the postnatal central and/or peripheral nervous system, including neurons and glia, even though these cells are non-dividing. Specificity may be achieved by anatomically specific delivery or by tissue specific expression.

Brief Summary Text (38):
The exogenous DNA preferably comprises a gene which encodes a gene product useful in the treatment of a nervous system disorder. This gene, in some embodiments, is operably linked to a promoter specific for particular cell types or regions within the nervous system. Because the AAV vector is integrated, stable, longterm expression (e.g., for greater than seven months) can be achieved.

Drawing Description Text (2):
FIG. 1 is a map of the AAV vector pAAVlac.

Drawing Description Text (3):
FIG. 2 is a schematic diagram outlining the relationship of the helper plasmid, AAV vector, adenovirus helper, etc.

Drawing Description Text (6):
FIG. 5 shows plasmid pAAV-FlagTH-AADC. This bicistronic construct contains the bicistronic construct with open reading frames for truncated tyrosine hydroxylase containing the N-terminal Flag epitope (Flag-TH) and aromatic amino acid decarboxylase (AADC). TH converts tyrosine to L-Dopa, and then AADC converts L-Dopa to dopamine. Between the two open reading frames is a sequence allowing ribosome re-entry and initiation of translation of a second open reading frame downstream from a translational stop codon. This is the internal ribosome entry site (IRES). These are transcribed as a single messenger RNA from the human cytomegalovirus immediate early gene promoter (CMV promoter). At the 3′ end of the insert is a signal for polyadenylation of the mRNA derived from the SV40 virus (SV40 polyA). The entire insert is flanked by terminal repeats from the adeno-associated virus (AAV term.), which permits replication, excision and packaging of the insert in the presence of proteins provided by the helper plasmid pAAV/Ad and helper adenovirus. The plasmid also contains standard plasmid sequences which permit replication and amplification of the DNA inside a bacterium (ori) and selection of bacterial colonies harboring the plasmid through resistance to ampicillin (amp). One

of the several unique features of the AAV vector is that unlike other defective viral vectors, these plasmid sequences are lost when the DNA between the AAV termini is packaged.

Detailed Description Text (5):
It is preferable that all of the viral genes be deleted, or otherwise inactivated, as in the known AAV vector dl3-94. However, it should be understood that a vector retaining one or more AAV genes such as the known AAV vector dl52-91, may still be useful for gene delivery, although inferior to the preferred vectors.

Detailed Description Text (6):
For propagation of the vector in vitro, susceptible cells are co-transfected with the AAV-derived vector and a suitable AAV-derived helper virus or plasmid. Preferably, the vector retains from AAV essentially only the recognition signals for replication and packaging.

Detailed Description Text (7):
It is not necessary that the AAV-derived sequences correspond exactly with their wild-type prototypes. For example, the AAV vectors of the present invention may feature mutated inverted terminal repeats, etc., provided that the vector can still be replicated and packaged with the assistance of helper virus, and still establish a nonpathogenic latent infection in target cells.

Detailed Description Text (9):
The AAV-derived Helper Virus or Plasmid

Detailed Description Text (10):
The AAV-derived helper virus or plasmid may be any virus or plasmid which is capable, upon expression of the carried AAV genes, of providing proteins necessary for the replication and packaging of the vector in vitro in a suitable host cell, for the purpose of producing vector stock.

Detailed Description Text (11):
In a preferred embodiment, the helper virus or plasmid is one which has been engineered to reduce the risk of recombination between the helper DNA and the vector DNA. Most desirably, there is essentially no sequence homology between the AAV sequences of the vector DNA, and the AAV sequences of the helper DNA. For example, the helper DNA may be an AAV in which the AAV inverted terminal repeats are replaced by the corresponding sequences of another virus, such as adenovirus (e.g., adenovirus type 5 DNA). See Samulski et al J. Virol. 63:3822-28.

Detailed Description Text (12):
Alternatively, in another preferred embodiment, helper adenovirus may be removed by heat inactivation at 56.degree. C. for 30 minutes, or separated from packaged AAV vectors by centrifugation in a cesium chloride gradient.

Detailed Description Text (15):
The "exogenous DNA" of the present invention should be exogenous to both AAV and to the target cell. The DNA may be synthetic DNA, complementary DNA, genomic DNA, or a combination thereof. The DNA

may be of any sequence or length, provided that it may be incorporated into the vector and delivered to target cells. Typically, because of the packaging limitations of AAV, the exogenous DNA will have a length of about 10-5,000 bases. Preferably, the DNA is 100 to 4,000 bases.

Detailed Description Text (24):
The above description of transducing striatal cells in vivo to a dopaminergic phenotype is the first step in a gene therapy approach to PD. However, PD becomes symptomatic when 80% of the dopamine neurons have been lost. Degeneration is progressive and with further denervation patients become increasingly refractive to all current therapies and exhibit "On-Off" phenomenon with increasing freezing and complete immobility. Transducing striatal cells with a viral vector to express dopamine synthesizing enzymes is purely a palliative approach and the underlying disease process will continue unabated. To this end vectors have been constructed expressing "neuroprotective or neurotrophic" factors to prevent further degeneration of dopaminergic neurons and promote regeneration. This approach includes the most specific neurotrophic factor for mesencephalic dopaminergic neurons identified to date, glial-derived neurotrophic factor (GDNF). Other neurotrophic factors of the NGF family have previously been expressed from HSV-1 vectors and shown to have neuroprotective effects (Federoff et al. These neurotrophic factors appear to act through tyrosine kinase receptors to prevent apoptosis or programmed cell death (PCD). As the proto-oncogene bcl-2 can prevent neuronal PCD in vitro, an AAV vector has been constructed expressing bcl-2 to prevent PCD in vivo. This vector might therefore be considered for any neuronal degeneration in the brain including ischemia, epilepsy or brain trauma where secondary neuronal injury occurs via PCD.

Detailed Description Text (25):
Therefore, gene therapy for PD could involve delivery, by AAV vectors, of the gene for GDNF (Genbank HUMGDNF02; Accession No. L19063), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) (EMBL HSNGF2; Accession No. X53655, and/or other members of the neurotrophin factor family including neurotrophin (NT)-3 (Genbank HUMBDNF; Accession No. M37762) and NT-4 (Genbank HUMPPNT4P; Accession No. M86528).

Detailed Description Text (26):
Recent evidence strongly implicates oxidative stress in the substantia nigra as a primary determinant of the progressive neuronal loss. Specifically, iron appears to be concentrated in the nigra of PD patients and studies have shown iron binding to neuromelanin of the nigra cells to generate free radicals. An antioxidant strategy to PD has therefore been proposed. The key enzymes which reduce free radical generation and/or scavenge free radicals are superoxide dismutase (SOD), catalase and glutathione peroxidase (GPO). Although mutations or alterations in expression of these enzymes has not yet been determined in PD, the increased expression of these enzymes in the nigrostriatal dopaminergic neurons will increase their ability to withstand oxidative stress. Therefore, an AAV vector has been made expressing SOD. This SOD expressing vector is also of interest in the treatment of amyotrophic lateral sclerosis (ALS) in which in the familial form

is associated with mutations in the SOD-1 gene.

Detailed Description Text (27):
Therefore, it may be desirable to use AAV vectors to deliver the genes for superoxide dismutase (SOD1 or SOD2) (GenBank HUMCUZNDI; Accession No. M12367; for SOD-1, EMBL HSSOD2G, Accession No. X65965 for SOD-2), catalase (EMBL HSCATR, Accession No. X04076), and/or glutathione peroxidase (MBL HSGSHPX, Accession No. Y00433).

Detailed Description Text (29):
Complex partial seizures and specifically temporal lobe epilepsy (TLE) is one of the most refractory forms of epilepsy. Although antiepileptic drugs (AED) will control seizures in some patients, 40% will remain uncontrolled despite polyAED therapy. The current approach for these patients is to undergo a phased evaluation for consideration of resective surgery. Typically, one temporal lobe is defined as the site of seizure origin (the epileptogenic region) and the medial temporal lobe including the anterior hippocampus is resected. TLE is not a genetic disease and there is no established aetiology, however the disease results from an imbalance of excitation to inhibition with interventions enhancing excitation or blocking inhibition producing seizures and conversely the antagonism of excitation and enhancing of inhibition has an antiepileptic effect. One gene therapy approach to TLE is to improve inhibition. To this end, the adenosine A-1 receptor (GenBank S56143; Accession S56143) cDNA has been inserted into the AAV vector. As adenosine has been shown to be the brain's natural anticonvulsant (acting through A-1 receptors) and levels of the receptor are decreased in the epileptogenic region, this strategy is likely to enhance inhibition and prevent seizures.

Detailed Description Text (33):
Therefore, for the treatment of epilepsy, genes encoding adenosine A-1 receptor (GenBank S56143; Accession S56143, glutamate decarboxylase (GenBank S61898; Accession S61898), GABA-A receptor isoforms (EMBL HSGABAAA1; Accession X14766), calcium-dependent potassium channels (GenBank DROKCHAN, Accession M96840) and/or ATP-sensitive potassium channels (Ho, et al 1993 Nature 362:31-8) may be delivered by AAV vectors.

Detailed Description Text (39):
This embodiment of the current invention envisions a significant improvement over these previous studies. Insertion of the TK gene (EMBL HEHSV1TK, Accession X03764; EMBL HEHS07, Accession V00466), into the AAV vector should permit transduction of genes into dividing tumor cells with efficiencies that are at least equal to retroviral vectors, and possibly with greater efficiency (which has been observed in comparisons of AAV and defective HSV vectors in rat brain). Unlike retroviruses, however, AAV vectors will also transfer the TK gene into slowly dividing or non-dividing cells within tumors as well as non-dividing normal cells. This could have two significant advantages compared with retroviral vectors.

Detailed Description Text (41):
The second advantage is the ability of AAV vectors to integrate in non-dividing cells. If a retrovirus enters a non-dividing cell, reverse transcription does not occur and the vector is lost. When

the AAV vector enters a non-dividing tumor cell, however, the vector should integrate into the host genome. Thus, if that tumor cell then re-enters cell division, the TK gene should be retained in that cell and all progeny. This should then render such previously quiescent tumor cells susceptible to destruction by ganciclovir or an analog. Since retroviral vectors are lost in non-dividing cells, and other DNA viral vectors do not reliably integrate within the host genome, the ability to retain the TK gene if a quiescent cell begins division is a property unique to the AAV vector. Finally, it should be reiterated that integration of the AAV vector should not result in disruption or abnormal regulation of host genes, and that transduction of normal non-dividing cells with TK should not have any adverse effects, since it is the subsequent activation of the drug by TK which blocks DNA replication and this only results in destruction of dividing cells. Thus, this embodiment of the invention provides substantial improvements over previous drug-susceptibility tumor treatment strategies.

Detailed Description Text (46):
To deliver the vector specifically to a particular region of the central nervous system, it may be administered by stereotaxic microinjection, as exemplified in Example 2. For example, on the day of surgery, patients will have the stereotactic frame base fixed in place (screwed into the skull). The brain with stereotactic frame base (MRI-compatible with fiducial markings) will be imaged using high resolution MRI. The MRI images will then be transferred to a computer which runs stereotactic software. A series of coronal, sagittal and axial images will be used to determine the target (site of AAV vector injection) and trajectory. The software directly translates the trajectory into 3 dimensional coordinates appropriate for the stereotactic frame. Burr holes are drilled above the entry site and the stereotactic apparatus positioned with the needle implanted at the given depth. The AAV vector will then be injected at the target sites. Since the AAV vector will integrate into the target cells, rather than producing viral particles, the subsequent spread of the vector will be minor, and mainly a function of passive diffusion from the site of injection, prior to integration. The degree of diffusion may be controlled by adjusting the ratio of vector to fluid carrier.

Detailed Description Text (52):
For targeting the vector to a particular type of cell, e.g., a neuron, it is necessary to associate the vector with a homing agent that binds specifically to a surface receptor of the cell. Thus, the vector may be conjugated to a ligand (e.g., enkephalin) for which certain nervous system cells have receptors. The conjugation may be covalent, e.g., a crosslinking agent such as glutaraldehyde, or noncovalent, e.g., the binding of an avidinated ligand to a biotinylated vector. Another form of covalent conjugation is provided by engineering the helper virus used to prepare the vector stock so that one of the encoded coat proteins is a chimera of a native AAV coat protein and a peptide or protein ligand, such that the ligand is exposed on the surface.

Detailed Description Text (53):
Whatever the form of conjugation, it is necessary that it not

substantially interfere either with the integration of the AAV vector, or with the binding of the ligand to the cellular receptor.

Detailed Description Text (76):
In addition to or instead of an expressible gene, the nucleic acid may comprise sequences homologous to genetic material of the target cell, whereby it may insert itself into the genome by homologous recombination, thereby displacing a coding or control sequence of a gene or deleting a gene altogether, provided that these sequences do not substantially interfere with integration of AAV.

Detailed Description Text (81):
Current approaches to transfer genes into the nervous system employ either recombinant viral vectors which retain viral genes or defective vectors containing residual and potentially dangerous helper viruses. Adeno-associated viral (AAV) vectors are non-pathogenic integrating DNA vectors in which all viral genes are removed (96% of the viral genome) and helper virus is completely eliminated. An AAV vector expressing .beta.-galactosidase was stereotactically injected into rat brain regions including striatum, hippocampus and substantia nigra. Vector DNA and transduced gene expression was detected from 1 day to 3 months post-injection. A second vector expressing human tyrosine hydroxylase (TH) was generated. This vector (AAVth) was injected into the denervated striatum of unilateral 6-hydroxydopamine-lesioned rats and TH immunoreactivity was obtained in striatal cells, including both glia and neurons, to 4 months. There was no evidence of pathology or toxicity in any animal treated with AAV vectors. Initial data indicates that TH transduction in the striatum via an AAV vector yields significant behavioral recovery in lesioned rats compared with AAVlac controls.

Detailed Description Text (83):
Plasmids: Plasmid pSub201 (Samulski et al (1989) J. Virol. 63:3822-28) was digested with XbaI to remove nearly the entire AAV genome, leaving only the terminal repeats. A CMV promoter-lacZ gene-SV40 polyA signal cassette was isolated from plasmid pHCL (Kaplitt et al (1991) Mol. Cell. Neurosci. 2:320-30) by digestion with SpeI and XbaI, and this was inserted into XbaI-digested pSub201 to create pAAVlac. A second plasmid was created (pAAV-CMV-polyA) by digestion of pAAVlac with HindIII and XbaI to remove the lacZ gene and polyA signal, followed by insertion of a HindIII-XbaI fragment from pREP4 (Invitrogen), containing a polylinker and SV40 polyA signal. This plasmid was then digested with HindIII and BamHI, followed by insertion of a human tyrosine hydroxylase (hTH) cDNA (O'Malley et al (1987) Biochemistry 26:6910-14) in order to create pAAVth.

Detailed Description Text (84):
Creation of Defective Viral Vectors: In order to create AAV vectors, plasmids (pAAVlac or pAAVth) were transfected via the calcium phosphate method (Graham et al (1973) Virology 52:496-67) into 293T cells, a variant of 293 cells (Graham et al (1977) J. Gen. Virol. 36:59-74), (obtained from D. Baltimore) which constitutively express both the adenovirus E1a protein and the SV40

T antigen. The vector plasmids were co-transfected along with the helper plasmid pAd8, which provides necessary replication and structural proteins. The next day, cells were infected with adenovirus strain dl309 (Jones and Shenk (1978) Cell 13:181-88) (obtained from Thomas Shenk, Princeton University). Following full cytopathic effect, virus was harvested by multiple freeze/thaw cycles. Viral stocks were then heated to 56.degree. C. for 30 minutes in order to inactivate residual adenovirus (Samulski et al 1989). Vector titers were obtained by histochemical assay for X-gal (Kaplitt et al (1991) Mol. Cell. Neurosci. 2:320-30) or immunocytochemical identification of hTH expression in 293T cells infected with serial dilutions of the vector stock, using a monoclonal anti-hTH antibody (Boehringer Mannheim) and the ABC elite detection system (Vector Labs).

Detailed Description Text (95):
Creation of an Adeno-associated Virus (AAV) Vector for Gene Transfer Into Brain

Detailed Description Text (96):
The bacterial lacZ gene was inserted into plasmid psub201 (Samulski et al (1989) J. Virol. 63:3822-28) between the termini of the AAV genome. These termini contain the recognition signals for cleavage and packaging into an AAV vector. The lacZ gene encodes the bacterial enzyme .beta.-galactosidase; which produces an insoluble blue precipitate upon reaction with the appropriate substrate. The human cytomegalovirus (CMV) immediate-early promoter was used to direct gene expression, and an SV40 polyadenylation signal was placed downstream of the lacZ gene (FIG. 1). Cells were transfected with pAAVlac and a second plasmid, pAd8 (Samulski et al. 1989), which provides AAV structural proteins but lacks AAV termini and thus cannot package into virus. Co-transfected cells were then infected with adenovirus type 5 (Jones and Shenk (1978) Cell 13:181-188) to provide remaining replication and packaging machinery (FIG. 2). The resulting stock consisted of packaged AAV-lac vectors (AAVlac) and progeny helper adenovirus; helper virus was then eliminated by heating at 56.degree. C. for 30 minutes. The complete elimination of adenovirus was confirmed by the inability to detect any viral plaques in cultured cells 1 week following infection with this viral stock. AAV vectors were then titered by infection of cultured 293 cells, histochemical staining for .beta.-galactosidase expression and counting of blue cells. There was no difference in the number of cells observed at 1 and 5 days following infection, demonstrating an absence of vector replication and spread. When the process was repeated using a lacZ plasmid without the AAV recognition signals, nb positive cells were observed following infection with the resulting stock. This indicates that the lacZ gene was packaged into an AAV virus which was incapable of autonomous replication while residual adenovirus was completely eliminated.

Detailed Description Text (98):
AAV Vectors Can Transfer and Stably Express A Foreign Gene in The Adult Rat Brain

Detailed Description Text (100):
In order to analyze the long-term stability of AAV gene transfer

and expression within the mammalian brain, animals were injected in the caudate nucleus with AAVlac and sacrificed 2-3 months following surgery. First, the polymerase chain reaction (PCR) was adapted for use within brain sections to permit amplification and visualization of viral vector DNA in situ. See Nuovo et al (1991) Am. J. Pathol. 139:1239-44; Nuovo et al (1993) PCR Meth, 2:305-12; Flotte et al (1993) Proc. Nat. Acad. Sci. USA 90:10613-17. Numerous cells within the brain were detected which retained the bacterial lacZ gene after 2 months. There was no staining on the opposite side of the brain sections or in sections processed without Taq polymerase, and positive cells were also absent from brains injected with adenovirus alone. Additional animals were then sacrificed and sections were processed for X-gal histochemistry (See Kaplitt et al (1991) Mol. Cell. Neurosci. 2:320-30) in order to identify cells containing functional .beta.-galactosidase. Positive cells were identified within injected regions of the caudate nucleus up to 3 months following vector injection. At no time were behavioral or physiological abnormalities detected within the animal subjects, and the brain sections showed no evidence of pathology resulting from the AAV gene transfer.

Detailed Description Text (102):
The AAV Vector Yields Expression of Tyrosine Hydroxylase in the Caudate Nucleus of 6-OHDA Lesioned Rats

Detailed Description Text (103):
Parkinson's Disease (PD) is a neurodegenerative disorder characterized by loss of the nigrostriatal pathway and is responsive to treatments which facilitate dopaminergic transmission in the caudate-putamen. (Yahr and Bergmann, Parkinson's Disease (Raven Press, 1987), Yahr et al. (1969) Arch. Neurol. 21:343-54. In experimental animals, genetically modified cells that express tyrosine hydroxylase, and thereby synthesize diihydroxyphenylalanine (L-Dopa), induce behavioral recovery in rodent models of PD. (Wolff et al. (1989) PNAS (USA) 86:9011-14; Freed et al (1990) Arch. Neurol. 47:505-12: Jiao et al (1993) Nature 262:4505). An alternative approach is that of direct in vivo somatic cell gene transfer whereby the intrinsic cells of the neostriatum are converted into L-Dopa-producing cells by transduction with a vector expressing TH. An HSV-1 vector expressing TH has shown that this approach may be a viable alternative to tissue transplantation. (During et al. (1992) Soc. Neurosci Abstr. 18:331-8). However, HSV-1 vectors currently have several limitations as described above. In order to generate a vector which may have therapeutic utility in human PD patients, we inserted a human TH cDNA (form II) (O'Malley et al. Biochemistry 26:6910-14) into our AAV vector (AAVth). AAVth was packaged and helper virus was eliminated as described above for AAVlac.

Detailed Description Text (105):
In order to examine virally encoded TH gene expression following transduction with AAVth, animals were analyzed at times ranging from 24 hours to 7 months after injection. Expression of TH from the AAV vector was detected using immunocytochemistry with a mouse monoclonal anti-TH antibody. Although this antibody does not distinguish between the rat and human protein, TH is not expressed within either the intrinsic neurons or glia of the rat striatum

(Chatterjee et al. (1992) Science 258:1485-88). Furthermore, endogenous TH immunoreactivity (TH-IR) within the striatum is limited to the dopaminergic afferent fibers in unlesioned animals and is absent in the completely denervated striatum. In both control and AAVlac-injected rats there was no striatal TH immunoreactivity (TH-IR) on the denervated side. In contrast, in the denervated striata injected with AAVth, numerous TH-IR cells were clustered around the injection site and extending to 2 mm away from the injection. The majority of cells within the striatum appeared to be neurons morphologically, and double-labelling with both the anti-TH monoclonal antibody and an anti-neurofilament antibody confirmed that a substantial number of intrinsic striatal neurons expressed immunoreactive TH de novo. Additional sections were then double labelled with both the TH monoclonal antibody and a rabbit polyclonal antibody to glial fibrillary acidic protein (GFAP), a marker of astrocytes and oligodendrocytes. Other sections were double labelled with TH antibody and antibodies for glutamic acid decarboxylase (GAD), a marker of GABAergic neurons, the predominant neuronal population of the neostriatum. Double labelling revealed that the majority of TH-IR cells were immunoreactive for GAD, while a small percentage of TH-IR cells were GFAP positive. GABAergic neurons constitute approximately 95% of the intrinsic striatal neuron population with choline acetyl transferase (ChAT, cholinergic) positive cells making up the remainder. Double labelling with TH and ChAT also revealed expression of vector encoded TH in striatal cholinergic neurons.

Detailed Description Text (106):
The titre of the AAVth stock used for these in vivo studies was 5.times.10.sup.6 infectious particles (i.p.)/ml. Therefore a single injection of 2 .mu.l would result in 10,000 positive cells if the efficiency of infection was 100% and each i.p. infected a different cell. However, as previous infection of AAV does not prevent subsequent re-infection or multiple particles infecting the same cell, in the immediate vicinity of the injection we might expect cells to have multiple infection. Moreover, AAVth might also infect axons and terminals and following retrograde transport be expressed in the cell body regions of the striatal afferents (e.g., the surviving dopamine nigral neurons, the cortex, reticular nucleus of the thalamus and dorsal raphe nuclei). In the AAVth-injected animals, the total number of striatal cells containing TH-IR consistently exceeded 1000 for each of the 2 .mu.l injections suggesting a minimum of 10% in vivo efficiency, significantly greater than previous observations using defective HSV-1 vectors (@ 2%). Moreover, the level of expression was also examined at times ranging from 3 days to 7 months. Expression persisted throughout this 7 month period, although the level of expression diminished by approximately 50%.

Detailed Description Text (109):
Expression of Two Genes From a Single AAV Vector Results in De Novo Synthesis of the Neurotransmitter Dopamine

Detailed Description Text (110):
Dopamine synthesis is catalyzed by two enzymes, TH and aromatic acid decarboxylase (AADC). The reaction catalyzed by TH results in the synthesis of L-Dopa, and this is the rate-limiting step in the

synthesis of dopamine. Dopamine then results from conversion of L-Dopa by AADC. Although striatum does not contain cells which endogenously produce TH, there are a small percentage of striatal cells which produce AADC. Therefore, behavioral recovery in animals treated with AAVth (or other approaches using TH alone) presumably occurs secondary to conversion of the resulting L-Dopa to dopamine by endogenous striatal AADC. Since a limited number of cells produce AADC, however, it is possible that synthesis of dopamine could be enhanced by expression of both TH and AADC in every transduced cell. In this manner, any target cell would become an autonomous dopamine-producing cell following gene transfer. Recent evidence in fact suggests that expression of both genes in the denervated striatum may be superior to expression of TH alone (Kang, et. al., 1993). Furthermore, the most substantial behavioral recovery following cell transplantation occurred when TH-expressing muscle cells were utilized (Jiao et al 1993), and unlike fibroblasts from earlier studies, muscle cells express endogenous AADC activity. This suggested that creation of an AAV vector containing both T14 and AADC would be valuable.

Detailed Description Text (111):
Due to the limitation on insert sizes in AAV vectors, several modifications were required in order to create a vector containing both genes. First, the TH gene was truncated, eliminating the 5' end. Truncation of the TH gene has actually been shown to increase enzymatic activity due to removal of an amino terminal regulatory domain. (Walker et al (1994) Bioch. Biophys. Acta 1206:113-119). Therefore, this served a functional purpose as well as increasing the space available for other genetic elements. In addition, a small synthetic oligonucleotide, encoding a novel epitope, was attached to the 5' end of the truncated TH. This novel epitope, termed "Flag", is recognized by a commercially available monoclonal antibody; this provides an independent and unambiguous marker for expression of AAV-transduced T14 in vivo.

Detailed Description Text (112):
After modifying TH, the AADC gene was inserted into the vector. Creation of two independent expression units, with two promoters and two polyadenylation signals, would have resulted in an insert size so large as to be incompatible with the constraints of the AAV vector. Therefore, an IRES element was inserted between the Flag-TH and AADC cDNAs. Most eukaryotic mRNAs are monocistronic; they contain a single-open reading frame, and when translation of a peptide is stopped and the ribosome falls off of the transcript, additional downstream translational start sites cannot be utilized. When the IRES element is present on an mRNA downstream of a translational stop codon, it directs ribosomal re-entry (Ghattas et al (1991) Mol. Cell. Biol. 11:5848-5959), which permits initiation of translation at the start of a second open reading frame (IRES=Internal Ribosome Entry Site). In this manner, a eukaryotic bicistronic mRNA can be created which allows translation of two distinct peptides from a single mRNA (FIG. 2). Thus, with only a single promoter (CMV) and a single mRNA polyadenylation signal (SV40) directing expression of a single transcript, translation of both the Flag-TH and AADC proteins could occur within a single cell transduced with a single AAV vector.

Detailed Description Text (113):
Following creation of the plasmid AAV Flag-TH/AADC, each of the independent expression parameters were tested in culture. The plasmid was transfected into 293T cells, and then the following day the substrate tyrosine and an essential co-factor (tetrahydrobiopterin) were added to the tissue culture medium of some of these cultures. For comparison, additional cells were transfected with the plasmid AAVlac or were mock-transfected. Samples of medium were obtained at 30 minutes and 60 minutes after addition of co-factors (or mock treatment), and these were analyzed for the presence of dopamine by high-performance liquid chromatography (HPLC). As indicated in FIG. 6, very high levels of dopamine were produced in 293T cells transfected with AAV Flag-TH/AADC in the presence of both co-factors. In similarly transfected cells lacking the co-factors, barely detectable amounts of dopamine were produced, while AAVlac-transfected or mock-transfected cells yielded absolutely no dopamine synthesis even in the presence of adequate co-factors. This indicated that 293T cells were incapable of endogenously directing dopamine synthesis, however introduction of the bicistronic vector AAV Flag-TH/AADC converted these cells into high level, co-factor dependent producers of dopamine. Finally, it should be noted that these cells were then fixed and stained with the anti-Flag monoclonal antibody, and this revealed highly specific histochemical detection of the Flag epitope with no background.

Detailed Description Text (114):
The specificity and function of the bicistronic AAV vector was further analyzed in cultured 293T cells. Despite the above data, it was still possible that 293T cells contained endogenous AADC activity. If this were true, then expression of Flag-TH alone would have yielded similar data without achieving translation of the second (AADC) open-reading frame. In order to test this, an additional vector was created. AAVFlag-TH contains a monocistronic insert with the Flag-TH open reading frame but lacking both the IRES sequence and the AADC open reading frame. 293T cells were then transfected with AAV Flag-TH/AADC, AAVFlag-TH, AAVlac or no plasmid. Both co-factors were added to all cultures the following day, and then samples of the medium were tested for both L-Dopa and dopamine by HPLC (Table 1). Cells which were transfected with no plasmid or AAVlac could not synthesize any-detectable level of either L-Dopa or dopamine. The lack of L-Dopa demonstrated that 293T cells do not possess any endogenous TH activity. Furthermore, cells transfected with AAVFlag-TH yielded very high levels of L-Dopa, but undetectable amounts of dopamine. This demonstrates that 293T cells do not possess any AADC activity either. Furthermore, this indicates that the truncated TH is highly active and the addition of the 5′ Flag sequence did not adversely influence enzymatic activity. Finally, cells transfected with AAV Flag-TH/AADC produced significant amounts of L-Dopa but very high levels of dopamine. Presumably the lower level of L-Dopa in these cells compared with those transfected with AAVFlag-TH was due to the efficient conversion of L-Dopa to dopamine. Thus two genes can be placed into a single AAV vector and techniques such as insertion of an intervening IRES sequence can result in translation of both protein products. These data also indicate that AAV vectors can yield expression of multiple, functionally active proteins which

can synergize in the production of a single, biologically active neurotransmitter. The Flag epitope was also shown to be a specific, independent marker of AAV-derived TH protein production without adversely influencing TH enzymatic activity.

Detailed Description Text (117):
AAV Vector-mediated Gene Therapy in a Primate Model of Parkinson's Disease

Detailed Description Text (119):
Early studies initiated in MPTP primates have been designed to test the safety of the AAV system in primates and to obtain information regarding the potential therapeutic efficacy of AAV Flag-TH/AADC for Parkinson's disease. The initial study employed a small number of animals with only moderate nigral lesions and was designed to determine whether AAV vectors can transfer genes in the adult primate brain, and whether dopamine transmission could be increased in the striatum using the bicistronic vector. Purified vector was stereotaxically injected unilaterally into the striatum of MPTP-treated primates, and subjects were then sacrificed either 10 days or 4.5 months after injection. Tissue sections were analyzed for Flag immunoreactivity, and numerous positive cells were demonstrated in several sections from the injected striatum in both short and long-term subjects, while sections from the uninjected side were completely negative.

Detailed Description Text (120):
The majority of positive cells appeared morphologically to be neurons. This demonstrated for the first time that AAV vectors could successfully transfer genes into the primate brain (During et al (1994) Abstr. Soc. Neurosci. 20:1465).

Detailed Description Text (121):
Biochemical analysis of tissue samples from treated primates. further indicated that the vector did cause an increase in striatal dopamine (During et al (1994)). For example, in one subject sacrificed at 10 days following treatment, the level of dopamine from a striatal tissue sample near the site of AAV injection was 18.93 ng/mg protein. An equivalent tissue sample from the uninjected, contralateral striatum yielded a dopamine level of 7.97 ng/mg protein. Tissue samples from distal sites on the injected and uninjected sides resulted in dopamine levels of 2.48 ng/mg and 2.27 ng/mg respectively. Since peripherally administered MPTP should result in roughly equal lesions to the substantia nigra bilaterally, the approximately 140% increase in dopamine levels in the injected striatum compared with the untreated side suggests that the AAV vector resulted in expression of functionally active enzymes.

Detailed Description Text (122):
A second study employed more severely lesioned primates in order to determine whether there is a therapeutic potential for AAV Flag-TH/AADC. Subjects were divided into two groups, with the treated group receiving AAV Flag-TH/AADC and controls receiving AAVlac. All animals received bilateral stereotaxic injections, with the same virus infused into the striatum on both sides of the brain. Subjects were then followed for 2.5 months after surgery.

Observations suggest that the bicistronic vector resulted in sustained improvement in parkinsonian behavior (During et al (1994). Monthly assessments of control and treated animals by blinded caretakers reported virtually no change in the behavior of animals which were subsequently determined to have been controls, while the response in treated subjects varied from modest improvement to substantial recovery of function. Most of the animals began the study unable, spending much of their time face-down and requiring assistance in order to feed and groom themselves. Reports indicate that improvements in treated animals resulted in some cases in decreased time spent face-down and recovery of the ability to feed and groom themselves. These blinded observations suggest that AAV vectors may result in behavioral recovery of parkinsonian primates. It should also be noted that in both primate studies, there was no behavioral or histological evidence of toxicity due to the AAV vector. All of the data indicate that safe, long-term improvement of human neurological diseases may be possible via genetic modification of adult brain cells in vivo using AAV vectors.

Detailed Description Text (124):
Expression of a Growth Factor From an AAV Vector Can Yield Recovery of Function Following Neuronal Lesions

Detailed Description Text (125):
An additional AAV vector has been developed as an alternative approach to the treatment of Parkinson's disease. To date, the majority of therapeutic strategies for PD have concentrated upon increasing striatal dopamine levels. Although behavioral recovery in animal models has been repeatedly demonstrated, this is not a cure for the disease but rather symptomatic palliation. Neuronal degeneration in the substantia nigra is the pathological result of the disease process, and progression of neurodegeneration is not altered by increasing striatal dopamine. Recently, however, several reports have determined that growth factors such as glial-derived neurotrophic factor (GDNF) can be protective of and trophic for neurons of the substantia nigra (Lin et al (1993) Science 260:1130-1132). Therefore, an AAV vector was created containing the cDNA for GDNF under the control of the CMV promoter.

Detailed Description Text (126):
Rats were lesioned with 6-OHDA and subsequently received injections AAVgdnf, AAVlac or saline into the lesioned substantia nigra (During et al (1994)). After several weeks, dopamine release into the striatum on the lesioned side was determined using intracerebral microdialysis. This technique permits sampling of local neurotransmitter release within a specific brain region of living animals (During and Spencer (1993) Lancet 341:1607-1610). Baseline dopamine levels were sampled three times and there was no difference between groups. Animals were then treated with potassium which induces release of dopamine from presynaptic terminals. Neither the AAVlac nor saline treated animals showed any variation in dopamine release from baseline, indicating that there were few dopaminergic terminals present within the striatum. The group treated with AAVgdnf, however, yielded an significant increase in dopamine release of 200% ($p<0.05$). Since the AAV vector only contained the gene for a growth factor, the restoration of

potassium-induced dopamine release into the striatum suggests that GDNF expression either protected or promoted regrowth of dopaminergic neurons in the substantia nigra following 6-OHDA treatment.

Detailed Description Text (127):
These results were further supported by subsequent administration of nomifensine to animal subjects after dopamine levels in the AAVgdnf group returned to baseline. Nomifensine is a drug which increases synaptic dopamine levels by inhibiting dopamine-reuptake. Again both control groups showed no change in dopamine levels in response to nomifensin, while striatal dopamine increased 150% ($p<0.05$) in the group treated with AAVgdnf. Together these data demonstrate that AAV-mediated transfer of a growth factor gene can either protect or restore dopaminergic inputs to the striatum. Thus gene therapy can be useful for both palliation of PD through striatal expression of synthetic enzymes for dopamine as well as for treatment of the underlying disease process by expressing growth factors which may protect or regenerate dopaminergic neurons. The present invention is the first demonstration that AAV vectors can safely and efficiently transfer and express a foreign gene marker gene (lacZ) in the adult rat brain. Furthermore, stability of viral DNA and lacZ expression within the brain was observed for at least 7 months with no evidence of pathology or toxicity. Expression of human tyrosine hydroxylase (hTH) was demonstrated in both neurons and glia of rat brains which had previously received unilateral 6-hydroxydopamine (6-OHDA) lesions in the substantia nigra. These lesions result in asymmetrical (contralateral to the side of the lesion) rotational behavior when rats are treated with apomorphine, and this has been used as a behavioral model of Parkinson's disease (PD). Following vector injection into the caudate nucleus, expression of hTH was demonstrated up to 7 months later, and preliminary evidence indicates that sustained expression of hTH from an AAV vector can reduce rotational behavior following 6-OHDA administration.